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LYMPHOCYTE CHANGES IN NORMAL APHERESIS DONORS

A thesis submitted to the

Division of Graduate Studies
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

at the Hoxworth Blood Center
of the College of Medicine

1983

by

Terry J. Meier

Captain, United States Air Force

B.S., University of Wisconsin-Madison, 1973

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I hereby recommend that the thesis prepared under my supervision by Terry J. Meier

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be accepted as fulfilling this part of the requirements for the degree of Master of Science

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THESIS ABSTRACT

LYMPHOCYTE CHANGES IN NORMAL APHERESIS DONORS

BY TERRY J. MEIER

CAPTAIN, UNITED STATES AIR FORCE,

BIOMEDICAL SCIENCE CORP

1983

For the degree of Master of Science
at the University of Cincinnati College of Medicine,
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Normal apheresis donors were studied to determine if any significant changes occur in the peripheral blood lymphocyte populations. Apheresis donors do have a significant reduction in the number of circulating B-cells following apheresis procedures. The long-term health effects of this B-lymphocyte loss is not known. No change in the ratio of helper to suppressor T-cells was observed when examined by monoclonal anti-human T-cell antibodies. Donor age and previous apheresis procedures did not appear to effect lymphocyte loss during apheresis. However, different apheresis instruments did show significant differences in the number of lymphocytes removed during normal apheresis procedures.

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First, I must express my appreciation to the apheresis donors that volunteered to participate in this study. Without them this investigation would not have been possible. Second, I must thank the staff of the apheresis unit for allowing me to interrupt their normal routine in recruiting and obtaining samples from the aphereis donors.

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If by chance there is any thing of value in this paper, the many people I have mentioned deserve the credit. If there are any mistakes, it's the computer's fault.

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Introduction

When platelet and granulocyte products are collected using automated apheresis equipment, they are accompanied by a certain number of donor lymphocytes. The primary goal of this research project was to study the effects of lymphocyte removal from normal donors undergoing apheresis. Concern has been expressed that lymphocyte removal could cause adverse changes in the donors' immune response.[1] Although there are no published reports that document any adverse changes in donor immune response following apheresis, lymphocyte depletion must still be considered a potential risk because of the following observations. First, the removal of lymphocytes via thoracic duct drainage or cytapheresis has been shown to suppress immune function in experimental studies.[2-7] Second, the thymus which provides the developmental microenvironment for T-lymphocytes is known to involute with age.[8] Third, some lymphocytes appear to be relatively long lived and may represent "immunological memory".[9] Fourth, changes in the ratios of the various T-lymphocyte subsets have been observed in certain immunological diseases.[10] Finally, some studies on plasmapheresis and apheresis donors have shown changes in serum immunoglobulin levels and circulating lymphocyte numbers.[11-14]

Lymphocyte depletion via thoracic duct drainage or

continuous flow centrifugation has been used in human experimental attempts to treat rheumatoid arthritis and to protect allograft transplantations. In these studies[2-7], a large number of lymphocytes (1.1 X 10^{10} to 3.0 X 10^{12}) are removed over a period of several days to weeks. Removal of at least 1.0 X 10^{11} lymphocytes is considered necessary to demonstrate improvement in arthritis patients.[6] Studies on these patients with massive lymphocyte depletion document the effects of lymphocyte removal. The observed effects include reduction or elimination of delayed and immediate hypersensitivity skin reactions, depressed immunoglobulin levels, increased survival times of allografts, changes in the architecture of the secondary lymphoid organs, and inability to produce a primary immune response.[4] It would appear that T-cell dependent cell mediated responses are more sensitive to lymphocyte depletion than humoral antibody responses since some investigators report significant depression of hypersensitivity and allograft rejection responses with minimal effects on circulating antibody levels.[4,5] Although apheresis donors are not subjected to the massive lymphocyte depletion used in these studies, the loss of lymphocytes during apheresis can be substantial. Dwyer et al.[12] report a loss of 2.2 to 3.9 X 10^9 T-lymphocytes per donation using intermittent flow centrifugation apheresis. Koepke et al.[11] report an average

loss of 3.5 X 10^9 lymphocytes per plateletpheresis procedure and calculated that this loss represented as much as 30% of the donors' circulating lymphocytes. It is not known if less intensive lymphocyte depletions have any significant effects on the donors' immune status. Furthermore, it is not known if there are cumulative effects resulting from multiple apheresis procedures.

Of further concern is the observation that the thymus involutes with age.[8] Studies on thymic biopsy specimens demonstrate a continual decline in the cellularity of the thymus with increasing age. Generally by 40 years of age, the thymus is completely involuted and can be considered non-functional. Since the thymus provides the microenvironment where T-lymphocytes develop, the ability for T-cell replacement in middle aged (ie. post-thymic involution) apheresis donors seems a pertinent question. It has been shown that age related changes do occur in the subtypes of T-lymphocytes present in the circulation.[16] It has been postulated that these changes in lymphocyte subtypes may be related to observed changes in the immune responsiveness that occurs with aging. There are no reports on the effects of lymphocyte removal and changes in lymphocyte subtypes in apheresis donors.

In addition to the age of the donor, the "age" of the lymphocytes removed must be considered. Estimates on the life span of human lymphocytes vary from 530 days to as long

as 10 years.[17-19] Most of these estimates have been made by studying chromosome aberrations produced by exposure to radiation. However, it must be kept in mind that there are certain difficulties in interpreting these studies.[20] First, not all of the lymphocytes are exposed to the same dose of radiation. As a result, only certain lymphocytes may be exposed dependent on their location within the body. In addition, some chromosome aberrations prevent cell division and serve as dependable cell markers, while other aberrations may allow division and therefore can be passed to daughter cells. Finally, these sublethally damaged cells may not have normal life spans. Nonetheless, it would appear that some lymphocyte types have relatively long life spans. This would be consistent with the concept of "immunologic memory cells" which forms the basis of secondary immune responses. The effect of lymphocyte removal during apheresis, possibly including some long-lived memory cells, is not known.

Additional consideration must be given to the potential for upsetting the mechanisms of immune regulation when lymphocytes are removed. Reinherz and Schlossman[10] have reviewed the recent data regarding changes in T-lymphocyte subsets in various disease conditions. Excess numbers of T4+ T-cells (helper/inducer) are associated with autoantibody formation and have been demonstrated in scleroderma, sarcoid

tumors, and Sjogren's syndrome. Reductions in T4+ lymphocytes are seen in some types of agammaglobulinemia and severe combined immunodeficiency disease. Changes in the relative numbers of T8+ T-cells (suppressors) also cause immunoregulation deficiencies. Reductions in T8+ lymphocytes are seen in some autoimmune diseases such as systemic lupus erythematosus, hemolytic anemia, multiple sclerosis, severe atopic eczema, inflammatory bowel disease and juvenile rheumatoid arthritis. In contrast, excessive numbers of T8+ suppressor cells are seen in immunodeficiency diseases. Patients with acquired agammaglobulinemia, recent viral infections and lepromatous leprosy appear to have excess activated T8+ suppressor cells. Furthermore, an inbalance in the ratio of helper to suppressor cells has been shown to cause decreased immunoglobulin production in vitro. The effect of cell removal on the balance of immunoregulatory lymphocytes has not been studied in apheresis donors.

Several studies have been conducted which specifically examine the effects of apheresis on donor lymphocytes. Most of the early studies focused primarily on the effect of plasmapheresis or plateletpheresis on serum protein or immunoglobulin levels.[21-23] In 1978, Mose[24] published data showing no significant changes in lymphocyte numbers or types (T or B cells) in donors undergoing as many as 40 double plasmapheresis procedures per year. Lichtiger and Trujillo[25] report no significant changes in the T-cell or B-

cell values in multiple plateletpheresis donors. [illegible] studies have reported significant effects on the lymphocyte populations of apheresis donors. Koepke et al.[11] demonstrated an average loss of 3.5 X 10^9 lymphocytes per plateletpheresis procedure (6 cycles on Haemonetics Model 30). When donors were subjected to weekly plateletpheresis for 10 weeks there was an average drop in absolute lymphocyte counts of 22%, although the relative differential leukocyte counts remained within normal limits. Half of the donors studied showed slight decreases in the number of B-cells which reverted to normal within one month following the last procedure. Ieromnimon et al.[13] reported on serum immunoglobulins and lymphocyte profiles in both normal whole blood donors and leukapheresis donors. Routine whole blood donation was found to have no effect on serum immunoglobulin levels or peripheral lymphocyte populations even in donors with as many as 100 life time donations. However, donors subjected to leukapheresis demonstrated several significant changes. These donors underwent 5 consecutive apheresis procedures at weekly intervals using 6 cycles on the Haemonetics Model 30. The mean number of lymphocytes removed per procedure was 8.0 X 10^9 (SD +/- 3 X 10^9). In all cases the serum IgG and IgM levels decreased significantly following apheresis and returned to normal within a week. Lymphocyte counts, however, showed

significant changes which did not revert to pre-apheresis levels 2 months following the last procedure. Although all lymphocyte types showed significant changes, the greatest depletion was of B-cells. Senhauser et al.[14] demonstrated similar changes in a retrospective study of cytapheresis donors that had undergone multiple apheresis procedures (average of 9 with a range of 6 to 17 procedures within one year). These donors showed a 14% mean difference in serum IgG when compared to a control population. Total absolute lymphocyte counts were 23% lower, T-cells were 25% lower and B-cells were 46% lower than the controls. Dwyer et al.[12] studied the effect of different types of apheresis procedures on circulating T-lymphocytes. They demonstrated a loss of 3.0×10^9 T-cells during plateletpheresis, and a 3.9×10^9 loss when hydroxyethyl starch (HES) and prednisone pre-medication were used for leukapheresis. If intravenous dexamethasone was used at the commencement of leukapheresis instead of prednisone pre-medication, the lymphocyte loss was 2.2×10^9. They also studied the mitogen response of lymphocytes following different apheresis procedures. The only significant change observed was an increased mitogen response in donors pre-medicated with prednisone.

This research project was intended to study two major areas. These include the actual lymphocyte loss in apheresis donors and the types of lymphocytes that are removed. In conjunction with this, the effects of donor age, type of

apheresis equipment, type of apheresis product, and previous apheresis donations on the lymphocyte loss were also investigated.

Material and Methods

Subjects and Apheresis Procedures

Donors were selected for the study based on age, sex, product, previous apheresis donations and apheresis instrument. An equal number of donors above 40 years old and under 40 years old were selected. In addition, males and females, and donors with and without previous apheresis donations were included. Furthermore, donations on three different instruments were studied. These included the IBM 2997 (International Business Machines Corporation, Princeton, N.J.), and the Haemonetics V-50 and Haemonetics Model 30 (Haemonetics Corporation, Natick, MA.) instruments. Finally, both plateletpheresis and leukapheresis donors were studied.

In addition to the apheresis donors, data were also collected on eleven individuals (normal controls). The controls included whole blood donors, blood center employees, and graduate students. Only persons that had never undergone apheresis were selected for the control group. The normal controls ranged in age from 19 to 54 years with six being under age 40 and five being over age 40. Six of the controls were male and five were female.

Apheresis donors were approached after completion of the physicial examination and medical history but prior to the start of the apheresis procedure. The research project was

explained and donors willing to participate were asked to sign a release form approved by the Human Research Committee of the University of Cincinnati Medical Center (Figure 1).

Collection of Specimens

Blood samples were obtained from the donors through the apheresis tubing set prior to connection of the line to the apheresis equipment (Pre-samples). When the apheresis procedure was complete, additional blood samples were collected from the line disconnected from the apheresis equipment before the needle was removed from the donors vein (Post-samples). Follow-up specimens were collected 7 to 10 days after the apheresis procedure (1 week samples).

The venous specimens collected pre-, post-, one week, and the specimens from the normal controls included two 10 ml tubes of heparinized blood and one 7 ml tube of ethylene diamino tetra-acetic acid (EDTA) anticoagulated blood. All blood specimens were collected using aseptic technique. In addition to the venous blood specimens, a 2 ml EDTA anticoagulated sample was obtained from the apheresis product in 13 of the apheresis procedures.

Separation of Lymphocytes from the Whole Blood

Two different procedures were used for lymphocyte separation. The first procedure was used on specimens collected from plateletpheresis donors, all 1 week follow-up specimens, and normal controls. Using 16X100 mm tubes,

approximately 5 ml of the heparinized whole blood was layered onto 3 ml of Lymphocyte Separation Media (LSM) obtained from Litton Bionetics (Kensington, MD.) The tubes were centrifuged at 750 X g in an Damon/IEC (Model HN-SII) centrifuge for 15 minutes. After centrifugation, the cells at the plasma-LSM interface were collected using a Pasteur pipet. This cell suspension was then centrifuged for 10 minutes at 750 X g in the Damon/IEC centrifuge to produce a cell button. The supernatant material was discarded and the cells used in subsequent tests.

The second separation procedure was used on the pre- and post- specimens obtained from leukapheresis donors. The use of pre-medication in these donors caused increased numbers of granulocytes in the samples and complicated the separation of lymphocytes. Approximately 5 ml of the heparinized whole blood was layered onto 5 ml of 1.072 gm/ml Ficoll-Hypaque[26] (Ficoll: Sigma Chemicals, St. Louis, MO. Hypaque: Winthrop Laboratories, New York, N.Y.) medium in 16X100 mm glass tubes. The tubes were then centrifuged for 15 minutes at 750 X g in the Damon/IEC centrifuge. The cells at the plasma-Ficoll-Hypaque interface were removed with a Pasteur pipet. This cell suspension was centrifuged for 10 minutes at 750 X g in the Damon/IEC centrifuge to produce a cell button. The supernatant material was removed and the cells were gently resuspended in 2 ml of pH 7.3phosphate-

buffered saline (PBS). The cell suspension was transfered to a 10X75 mm glass tube. Clumping of cells was visible in the cell suspension macroscopically and examination of crystal violet stained material showed the clumped cells to be granulocytes. The cell suspensions were centrifuged for 2 seconds at 200 X g to remove the clumped cells (quick spin). The supernatant material was removed and placed in a clean 10X75 mm tube. This tube was then centrifuged for 10 minutes at 750 X g and the supernatant removed and discarded. The cells were resuspended in 2 ml of PBS. If clumping was still visible, the quick spin procedure was repeated. The quick spin procedure followed by removal and centrifugation of the supernatant was repeated until no clumping was visible in the cell suspension. These cell suspensions were then used in subsequent tests.

Determination of T-Cell Subsets

Commercial monoclonal mouse anti-human antibodies, OKT3, OKT4 and OKT8 (Ortho Diagnostics, Raritan, N.J.) were used to determine the types of T-lymphocytes present in the blood specimens.[27] The OKT3 antibody reacts with more than 95% of peripheral T-lymphocytes and is used to identify and enumerate human T-cells. Approximately 75% of the peripheral lymphocytes are T-cells. The OKT4 antibody reacts with 65% of peripheral T-lymphocytes (or 48.8% of the total peripheral lymphocytes) and identifies the helper/inducer T-

cell population. The OKT8 antibody reacts with 35% of the peripheral T-lymphocytes (or 26.3% of the total peripheral lymphocytes) and identifies the suppressor/cytotoxic T-lymphocytes[28]. These monoclonal antibodies were received lyophilized, rehydrated according to the package insert, and stored frozen in 0.01 ml aliqouts.[29]

An aliquot of the cells obtained from the lymphocyte separation procedure previously described was washed in 15 ml of cold (4 C) PBS. The suspension was centrifuged for 10 minutes at 900 X g in a refrigerated (4 C) Beckman TJ-6 centrifuge (Beckman Instruments, Palo Alto, CA.). After removing the supernatant material, the cells were resuspended in 2 ml of a red cell lysing solution (0.15M NH_4Cl with 0.0001M EDTA in PBS).[29] The suspension was vigorously agitated and allowed to incubate at room temperature for 10 minutes. After incubation, the cells were washed twice in 15 ml of cold PBS with centrifugation in the refrigerated Beckman centrifuge as outlined above. After the second wash, the supernatant material was completely decanted and the cells resuspended in 2 ml of PBS. A 1:10 dilution of the cell suspension was made using a white cell diluting pipet and the cell concentration determined using a hemacytometer. The cell concentration was adjusted by dilution with PBS to 1 $X10^7$ cells per ml. Four 0.1 ml aliquots of the adjusted cell suspension were transfered to four 10X75 mm glass tubes numbered 1 to 4. The OKT3 antibody

(0.01ml) was added to tube #1. Similarly, tubes #2, 3, and 4 received 0.01 ml of OKT4, OKT8 and PBS respectively. The tubes were then mixed and incubated in a wet ice bath for 30 minutes. After incubation, the cells were washed in 2 ml of cold PBS with centrifugation in the refrigerated Beckman centrifuge at 900 X g for 10 minutes. The supernatant PBS was decanted and 0.1 ml of a fluorescein-labeled goat anti-mouse immunoglobulin (Cappel Labs, Cochranville, PA.) was added. The working dilution of the goat anti-mouse immunoglobulin reagent had been previously determined to be 1:5 in PBS. The tubes were mixed and incubated in a melting ice bath for 30 minutes. After incubation, the cells were washed in 2 ml of cold PBS, centrifuged in the Beckman centrifuge at 4 C, and decanted to dry cell buttons. Two drops of slide mounting medium (30 per cent v/v glycerol in PBS) were added to each tube. The suspensions were mixed and one drop was placed on a glass slide. A cover slip was placed over the drop and the edges of the cover slip were sealed with clear fingernail polish.

Each slide was examined using a Leitz Diavert microscope (E. Leitz Inc., Rockleigh, N.J.) equipped for both phase and fluorescent microscopy. The slides were examined using a magnification of 500 X. The fluorescent microscopy was done using a PloemoPak I-2 filter block with a blue 450 to 490 nm exciting filter, a 515 nm suppression filter, and a mercury

light source. A total cell count per field examined was obtained using phase illumination. Only cells that appeared to be lymphocytes were included in the total cell counts. The microscope was then switched to fluorescent illumination and the number of cells with membrane fluorescence was determined. This procedure was repeated on additional fields until a total cell count of 200 was reached. The per cent of cells positive for a given monoclonal label was determined as follows:

Number of fluorescent cells divided by Total cell count =

Per Cent of cells positive for antigen

This procedure was carried out for each slide (OKT3, OKT4, OKT8 and Cell Control). If the Cell Control slide showed more than 2% positive cells, the results were considered invalid and the labeling procedure was repeated.

Determination of B-lymphocytes

An aliquot of the cells obtained in the lymphocyte separation procedure was washed in 2 ml of 37 C Roswell Park Memorial Institute medium # 1640 (RPMI). After removing the supernatant material, the cells were resuspended in 2 ml of a red cell lysing solution (0.15M NH_4Cl with 0.0001M EDTA in PBS). The suspension was vigorously agitated and allowed to incubate at room temperature for 10 minutes. The suspension was centrifuged at 750 X g for 10 minutes in the Damon/IEC centrifuge. The supernatant material was decanted and the

cells were washed twice with 2 ml volumes of 37 C RPMI #1640 medium. After the final wash, the cells were resuspended in 0.2 ml of the culture medium and the number of cells in the suspension was determined by diluting 1:10 in a white cell diluting pipet and counting the diluted material in a hemacytometer. The cell suspension was adjusted to 1 X 10^7 cells per ml by dilution with RPMI #1640 medium. A 0.1 ml aliquot of the adjusted cell suspension was transfered to a 10X75 mm glass test tube and 0.01 ml of a 2% (v/v) latex bead (Difco Laboratories, Detroit, MI.) suspension was added. The suspension was then mixed and incubated for 30 minutes at 37 C. The cells were then washed in 2 ml of 37 C RPMI #1640 medium and then washed again in 2 ml of room temperature PBS. The centrifugation for all washes was at room temperature for 10 minutes at 750 X g in the Damon/IEC centrifuge. The PBS supernatant was decanted and 0.1 ml of a fluorescein-labeled FAB fragment goat anti-human immunoglobulin (Cappel Labs Cochranville, PA.) was added. The working dilution of the fluorescein-labeled goat anti-human immunoglobulin had been previously determined to be 1:4. The suspension was then incubated in a melting ice bath for 30 minutes. After incubation, the cells were washed in 2 ml of cold PBS and centrifuged for 10 minutes at 900 X g in a refrigerated (4 C) centrifuge. The supernatant material was decanted and two drops of slide mounting medium were added to the tube. One drop of the cell suspension was

placed on a glass slide, covered with a cover slip, and sealed with clear fingernail polish. The slides were examined using the same microscope described for the T-cell subset determinations. Once again, total cell counts were performed using phase illumination and membrane fluorescence determined using fluorescent illumination. Cells that had ingested latex particles (monocytes) were not included in the total cell count or fluorescent cell count. The per cent of B-cells was calculated as follows:

Number of cells fluorescent divided by Total cells counted =

Per Cent B-Cells

Determination of Total White Cell Count and Absolute Lymphocyte Count

The EDTA anticoagulated specimens were used to determine the donors' white cell count and absolute lymphocyte count. Total white cell counts were performed using the Unopette system (Becton-Dickinson, Rutherford, N.J.). With this system, 0.02 ml of the anticoagulated blood is diluted 1:100 in a 1% buffered ammonium oxalate solution. The red blood cells are allowed to lyse and the white cell count determined using a hemacytometer.[30] Thin smears of the EDTA blood were also prepared on glass slides. The smears were stained with Wright's stain and standard 100 cell differential counts were performed. The absolute lymphocyte

count was calculated as follows:

Total White Cell Count X % Lymphocytes in Differential Count = Absolute Lymphocyte Count in Cells per mm^3

Statistical Analysis of Data

Statistical analysis of the data was performed using Student's t test for small sample inferences. A paired difference t test (paired t test) was used to compare data within one group. For comparison of data between groups, the difference between the means was used (unpaired t test).[31] The t test was interpreted as a two-tailed test at the $p=0.05$ ($t=0.025$ or 95% level) unless otherwise stated. Values of the calculated t test that fell below the critical value of t from tables were considered not significant (n.s.).

Results

Data were collected on 15 different apheresis donors (see Figure 2). One donor (donor #1) was studied on two different occasions. The donors ranged in age from 21 to 55 years with seven of the donors being under age 40 and eight being over age 40. Eleven of the donors were males and four were females. This was the first apheresis procedure for eight of the donors. Eight other donors had previous apheresis procedures. Six of the donors underwent plateletpheresis on the IBM 2997 Blood Cell Separator. Four donors underwent plateletpheresis and two donors underwent leukapheresis (granulocytes) on the Haemonetics Model 30 Blood Processor. One of the Model 30 platelet procedures involved the preparation of a red blood cell free product. Both of the granulocyte donors were pre-medicated, one with oral prednisone and the other with intravenous dexamethasone. Both leukapheresis procedures included the use of hydroxyethyl starch (HES). Four of the donors underwent plateletpheresis on the Haemonetics Model V-50 Blood Processor.

The mean and standard deviations for the T-cell subset and B-cell results on the eleven normal controls are presented in Table 1. The normal controls were included in this study to determine if the T-cell subset and B-cell procedures were producing reliable results. The mean values

obtained for the T-cell subsets and T4:T8 ratios for the normal controls were not significantly different from the values published in the Ortho Diagnostics package insert.[29] Furthermore, the results were comparable to published results obtained by other investigators using the same monoclonal antibodies and similar methods.[32-33] Likewise, the B-cell results were comparable to published results using similar materials and methods.[34-37]

The results for the donors' total white cell counts and absolute lymphocyte counts are shown in Tables 2a through 2d. There was no statistical difference in the pre- versus post- white cell counts in the IBM 2997 platelet donors (Table 2a), the Haemonetics V-50 platelet donors (Table 2b) or the Haemonetics 30 leukocyte donors (Table 2c). These donors also showed no statistically significant difference in their pre- versus post- absolute lymphocyte counts. Some of the donors did not return for the one week follow-up specimens, therefore the one week data was not available (NA). Statistical analysis of the one week specimens was not determined (ND) because of the small number of samples in each group. The Haemonetics 30 platelet donors did show a significant ($p=0.05$) decrease in their total white cell counts (Table 2d). The absolute lymphocyte counts in these donors also decreased. However, this decrease was significant only at the $p=0.10$ level. Comparison of the post- samples with the one week samples for the

Haemonetics 30 donors showed no statistically significant difference in the total white cell counts or absolute lymphocyte counts. Cumulative results for all apheresis donors compared using the unpaired t test are presented in Table 2e. No statistically significant differences were observed between the pre- and post- or post- and one week specimens.

Results for the white cell counts, total white cells, per cent lymphocytes, and total lymphocytes in the apheresis products are presented in Tables 3a through 3d. The IBM 2997 products had the lowest total white cells with a mean of 7.3×10^7 cells (Table 3a). The mean for the Haemonetics V-50 products was 3.7×10^8 (Table 3b). The Haemonetics 30 had the highest total white cells of all the platelet products with a mean of 4.5×10^9 cells per product (Table 3d). Comparison of the different platelet products showed that there was a significant difference between the total lymphocytes present (Table 3e and Figure 3). The differences between the IBM 2997 and Haemonetics V-50 and the differences between the IBM 2297 and Haemonetics 30 were significant at the p=0.05 level. The difference between the Haemonetics V-50 and Haemonetics 30 were significant only at the p=0.10 level.

The T4 (helper) and T8 (suppressor) subset results are presented in Tables 4a through 4e along with the calculated

T4:T8 ratios. Analysis using the paired t test on the T4, T8, and T4:T8 ratios results for the IBM 2997 platelet donors (Table 4a), the Haemonetics V-50 platelet donors (Table 4b) and the Haemonetics 30 leukocyte donors (Table 4c) showed no significant difference between the pre- and post- samples. Because of the small sample size, the one week values were not evaluated. The pre- versus post- and post- versus one week comparisons on the Haemonetics 30 platelet donors showed no significant differences. The T3, T4, and T8 data for all the apheresis donors was combined and evaluated using the unpaired t test (Table 4e). Again, there was no statistically significant difference between the pre- and post- values or the post- and one week values.

The results for the monoclonal T3 antibody are presented in Tables 5a through 5e. In addition to the percent of cells positive for the T3 antigen, a calculation of the absolute T-cell numbers is also presented. The absolute T-cell values were calculated by multiplying the percent of T-cells positive for the T3 antigen times the absolute lymphocyte count from Tables 2a through 2d. The IBM 2997 plateletpheresis donors showed no significant change in the percent of T-cells or in absolute T-cell numbers (Table 5a). Similarly, no changes were observed in the pre- and post- values for the Haemonetics V-50 donors. Analysis of the Haemonetics 30 leukapheresis donors was not possible because of the small sample size (Table 5c). The Haemonetics 30

plateletpheresis donors showed no significant difference between the pre- and post- values or the post- and one week values (Table 5d). The results for all the apheresis donors was cumulated and analyzed using the unpaired t test (Table 5e). Again, no significnat differences were observed between the pre- and post- data or the post- and one week values.

The results for the B-cell determinations are presented in Tables 6a through 6e. An absolute B-cell count was calculated using the percentage of B-cells and the absolute lymphocyte counts from Tables 2a through 2d. When the pre- and post- percentage of B-cells was compared using the paired t test, significant ($p=0.05$) changes were observed in the IBM 2997 donors (Table 6a), and the Haemonetics V-50 donors (Table 6b). However, the change in the absolute B-cells numbers was not significant for these donors. The Haemonetics 30 platelet donors also showed a drop in the percentage of B-cells after apheresis, but the results were significant only at the $p=0.10$ level (Table 6d). In contrast to the IBM 2997 and Haemonetics V-50 results, the reduction in absolute B-cell numbers was significant for the Haemonetics 30 donors. There was no significant difference between the post- and one week values on the Haemonetics 30 donors. This comparison could not be done on the other donors because of the small number of samples in each group.

B-cell data were available for only one of the two Haemonetics 30 leukocyte donors, therefore statistical analysis was not possible (Table 6c). The cumulative results for all of the donors are presented in Table 6e and Figure 4. Analysis of these results using the unpaired t test showed no difference between the pre- and post- values or the post- and one week values for percent B-cells or absolute B-cell numbers at the p=.05 level. However, the reduction in absolute B-cell numbers was significant at the p=.10 level for the pre- versus post- comparison.

To determine if the donors' age had any effect on the T-cell subset or B-cell values, the data were divided into two groups; under 40 and over 40 years of age. Within each group, comparisons were made between the pre- and post-apheresis data (Table 7a). There was no statistical difference in the pre- versus post- T3, T4, T8, T4:T8 ratio, or B-cell values. In addition, the post- data between the two groups were also compared (Table 7b). No statistical difference was detected between the values for the under 40 and over 40 donors. A comparison of the total white cell counts and absolute lymphocyte counts in the two age groups was also performed using the unpaired t test. There was no significant difference in the pre- and post- total white cell counts (t=0.748), the post- and one week total white cell counts (t=0.748), the pre- and post- absolute lymphocyte counts (t=0.950) or the post- and one week

absolute lymphocyte counts in the over 40 year group. Analysis of the values for the under 40 group also showed no significant differences in: pre- versus post- total white cells (t=0.548), post- versus one week total white cells (t=0.966), pre- versus post- absolute lymphocytes (t=0.593), post- versus one week absolute lymphocytes (t=0.317). Finally, analysis of the post- data between the two groups showed no siginificant difference in the total white cell counts or absolute lymphocyte counts: post- over 40 versus post- under 40 total white cells (t=0.501), post- over 40 versus post- under 40 absolute lymphocyte counts (t=0.285).

The data also were divided into two groups based on the donors' previous apheresis history. For seven of the donors, this was their first apheresis procedure. Eight of the donors had a history of previous apheresis donations (see Figure 2). When the pre- and post- T3, T4, T8, T4:T8 and B-cell values were compared within the two groups, no statistically significant differences were observed (Table 8a). Similarly, comparison of the post- values between the two groups revealed no statistical difference (Table 8b). A comparison of the post- absolute lymphcyte counts between the two groups using the unpaired t test did not show any significant difference in the response to apheresis with respect to lymphocyte loss (t=0.247).

Discussion

The only significant decrease in the donors' total white cell counts or absolute lymphocyte counts was observed in the Haemonetics 30 plateletpheresis donors. This correlates with the observation that the Haemonetics 30 had the highest mean total lymphocyte counts of all the products studied. Many of the published studies on lymphocyte changes in apheresis donors utilized the Haemonetics 30 apheresis equipment.[11-14] This raises the possibility that the published literature on this subject may over emphasize the lymphocyte depletion problem. The other apheresis instruments in this study did not produce any significant changes in the donors' white cell or absolute lymphocyte counts. Furthermore, the lymphocyte contamination in the products from these instruments was significantly lower than the lymphocyte contamination in the Haemonetics 30 products. These newer technology apheresis instruments appear to be producing products with lower levels of lymphocyte contamination[38], a fact which should be taken into account when evaluating lymphocyte depletion in apheresis donors. As many as 1 X 10^9 lymphocytes may be removed during a whole blood donation. No adverse effects have been reported even after 40 years of donating whole blood.[39] The lymphocyte

loss per donation using the newer technology apheresis equipment is comparable to the loss in a single whole blood donation.

One of the Haemonetics 30 plateletpheresis donors had substantially lower white cell and total lymphocyte contamination present in the product (Donor #12 Table 3d). The procedure on this donor was modified to produce a product free from red blood cell contamination. With the modified procedure, the operator makes the "cut" before the instrument begins to harvest the red blood cell layer. Generally, an additional two cycles of collection are necessary to produce adequate platelet yields using this technique. It would appear that the modification reduced the white cell contamination as well as the red cell contamination. The Haemonetics V-50 instrument automatically stops the harvest of platelets when red blood cell contamination is detected by optical sensors. The operator of the IBM 2997 instrument optically controls the position of the platelet collection port so that it remains well above the red blood cell layer during the procedure. This correlates with the observation that the IBM 2997 and Haemonetics V-50 products are relatively red cell free (visually at least). As noted in this study, both these machines produced products with reduced white cell contamination. It would appear that a modification of the collection procedure on the Haemonetics 30 might also reduce

donor lymphocyte loss. It must be noted however, that the modified procedure is more time consuming and therefore may be unacceptable to some apheresis centers as well as some donors.

Wright et al.[7] studied the effects of blood flow rate and centrifuge speed on lymphocyte removal during apheresis. They showed that the T and B lymphocytes tend to position themselves at the plasma-blood cell interface. The T-cells were more concentrated on the plasma side of the interface. In contrast, the B-cells tended to collect on the cell side of the interface. The maximal T-cell concentration occured within the interface layer with a 2.0 to 3.0 gm/dl hemoglobulin level. These results would tend to confirm the observation that red cell contamination correlates with lymphocyte contamination. Wright et al.[7] also showed that the blood flow rate and centrifuge speed could significantly influence the numbers of lymphocytes collected. This raises the possibility for altering operating techniques for the different apheresis instruments to minimize donor lymphocyte loss.

The mean total lymphocyte loss for all apheresis instruments in this study was 1.3 X 10^9 cells. The Haemonetics 30 was highest with a mean of 2.5 X 10^9 cells. These values are in general agreement with the published results for the Haemonetics 30 equipment.

Ieromnimon et al.[13] reported a mean count per procedure of 8×10^9 (+/- 3×10^9) of white cells with 77% (+/- 14%) being lymphocytes. These results were observed during leukapheresis without pre-medication or HES. The mean lymphocyte loss was 6×10^9 per procedure. Dwyer et al.[12] report a mean loss of 4.6×10^9 lymphocytes per platelet procedure and 5.2×10^9 per leukapheresis using pre-medication and HES. Senhauser et al.[14] reported mean counts of 3.6×10^9 for leukapheresis and 4.0×10^9 for plateletpheresis.

As noted, only the Haemonetics 30 donors showed significant changes in peripheral blood absolute lymphcyte counts in this study. Other investigators have reported more significant changes than those observed in this study. Dwyer et al.[12] reported an average decrease of 24%, Koepke et al.[11] a 22% decrease and Ieromnimon et al.[13] a 18% decrease when measurements were made pre- and post-apheresis. Senhauser et al.[14] reported a 23% lower absolute lymphocyte count in apheresis donors compared to a normal (non-apheresed) population. However, in most of these studies, the donors were subjected to intense apheresis protocols over relatively short periods of time. In the Ierominmon et al.[13] study the donors were leukapheresed five times in a five week period. Koepke et al.[11] performed plateletpheresis procedures 10 times over a 12 week period on the same donors. The report of Senhauser et al.[14] was on

donors that had undergone 9 apheresis procedures within a 12 month period. Only the study of Dwyer et al.[12] involved random apheresis donors. In a study of apheresis on rheumatoid arthritis patients and normal controls, Wright et al.[7] concluded that removal of at least 1 X 10^9 lymphocytes per day over several days was necessary to produce significant declines in peripheral blood lymphocyte counts. Normal controls subjected to three or more leukapheresis procedures per month showed no consistent drop in their lymphocyte counts if the cell loss per procedure was less than 1 X 10^9 lymphocytes.

Only two of the donors in this study had multiple apheresis procedures over a short period of time. Donor #2 had undergone 6 previous apheresis procedures during the preceeding six months. The results for this donor showed no significant drop in white count or absolute lymphocyte count during the studied procedure. In fact, the absolute lymphocyte count in this donor actually increased slightly after apheresis (Table 2a). All of the apheresis procedures on this donor were performed on the IBM 2997 instrument. Donor #3 underwent 7 plateletpheresis procedures over a ten month period. These procedures were performed on different instruments including the IBM 2997, Haemonetics V-50 and Haemonetics 30. This donor did experience a 26% drop in absolute lymphocyte count after the studied apheresis. There

was a substantial age difference between these two donors (Donor #2 29 years vs Donor #3 54 years). The significance of this observation is unclear. Ieromnimon et al.[13] and Senhauser et al.[14] did not report the ages of their apheresis subjects. The mean age of the subjects in the Dwyer et al.[12] study was 36 years, but the range and distribution were not given. The Koepke et al.[11] study was of 10 donors between the ages of 21 and 36 with a mean of 26 years. The normal controls used in the Wright et al.[7] study were from 26 to 34 years of age. It is immpossible to determine if the results observed in the two multiple apheresis donors in this study are related to age or apheresis instrument(s) used. The younger donor had been apheresed only on the IBM 2997. Perhaps the low level of lymphocyte removal with this instrument prevented lymphocyte depletion in this donor. The older donor had been apheresed on several different instruments. The decrease in absolute lymphocyte count observed in this donor might be due to previous lymphocyte reductions, or could be age related.

In this study no significant changes were observed in the number of T-lymphocytes in the donors' peripheral blood when measured by either percent of cells positive for the T3 antigen or by absolute T-cell numbers. Koepke et al.[11] also reported no change in the peripheral blood T-cell numbers when measured by the sheep cell rosetting technique. Iermnimon et al.[13] showed a small decrease in the number of

T-cells after the first leukapheresis using a modified sheep cell rosetting technique. This drop in T-cells escalated after additional leukapheresis procedures. Dwyer et al.[12] did not observe a significant decrease in the T-cell numbers in plateletpheresis or leukapheresis donors. They also used the rosetting method. Senhauser et al.[14] reported 25% lower T-cell values in apheresis donors compared to normal controls with the rosetting technique. They did not however, measure pre- and post-apheresis T-cell levels.

The majority of peripheral blood lymphocytes are T-cells. Although all of the above investigators report reductions in the absolute lymphocytes counts, they fail to agree on the changes to the donors peripheral T-cell numbers. Dwyer et al.[12] showed that over 60% of the lymphocytes present in plateletpheresis products were T-cells. They calculated the loss to be 3 X 10^9 T-cells out of a total lymphocyte count of 4.6 X 10^9 cells. Since the T-cells seem to be the major population removed, it is odd that more pronounced changes are not observed in the peripheral blood of the donors. It can be speculated that lymphocytes are recruited from the secondary lymphoid organs and thoracic duct to replace those removed during apheresis. If this is indeed the case, it would appear that the response time is fairly short since no differences were observed in this study with specimens collected pre-

apheresis and immediately after the apheresis procedure.

If there is a rapid replacement of lymphocytes removed during apheresis, the ratios of helper to suppressor replacement cells must be similar to the normal ratio in the peripheral blood. No significant changes were observed in the T4(helper), T8 (suppressor) or T4:T8 ratios after apheresis. This observation also suggests that the helper and supressor cells are removed in proportion to their occurrence in the peripheral blood. That is, there is no preferential removal of any one subset of cells. This being the case, there would be little chance of any upset in the immunoregulatory balance of the immune system following apheresis, at least with respect to T-cell regulated functions.

B-cells however, do appear to be preferentially removed. Many investigators have demonstrated the "B-cell lesion" in apheresis donors. Koepke et al.[11] reported that 5 out of 10 plateletpheresis donors studied showed significant, although slight, reductions in peripheral blood B-cells. Follow-up study on these donors showed that the B-cell levels had returned to pre-apheresis values after one month. Ieromnimon et al.[13] report a 61% drop in B-cells after 5 leukapheresis procedures. Their follow-up study showed that the values had increased, but not to pre-apheresis levels, after two months. Senhauser et al.[14] compared B-cell levels in multiple apheresis donors to a control population. The

apheresis donors had a 46% lower mean B-cell level than the controls. The results in this study confirm these earlier findings. Statistically significant reductions of the percentage of B-cells did occur in the apheresis donors with no evidence of recovery at one week post-apheresis. However, a significant drop in the absolute B-cell numbers was detected only in the Haemonetics 30 platelet donors.

Since the B-cells represent a small proportion of the peripheral blood lymphocytes (about 10%), it would appear that they are being removed at a higher rate than T-cells. The reason for this observation may be related to the surface properties of B-cells. B-Cells are known to be "sticky" and will adhere more readily to glass or plastic surfaces.[40] It seems possible that during apheresis the B-cells adhere to the plastic surfaces in the tubing, apheresis bowls and transfer packs. There are no published studies of B-cell numbers in apheresis products. If the above theory is correct, the products may not have increased percentages of B-cells since most of the B-cells would be attached to the disposable plastic apheresis equipment. An alternate explanation would be that the B-cells are less able to migrate from the secondary lymphoid organs and thoracic duct and therefore respond to peripheral removal at a slower rate.

Obtaining leukocyte donors for use in this study proved

to be difficult. The number of requests for granulocyte products has been declining at this blood center, and only a limited number of granulocyte procedures were done during the time the study was being conducted. Separation of the lymphocytes from the granulocytes was an additional difficulty. The LSM, with a specific gravity of 1.077 to 1.080, failed to separate the lymphocytes from the large number of granulocytes present in the blood of the pre-medicated donors. The use of a lower specific gravity (1.072) Ficoll-Hypaque solution was attempted in hopes that the excess granulocytes could be driven through the lower density material.[26] The "quick spins" were also used to remove contaminating granulocytes by taking advantage of the tendency for the granulocytes to spontaneously agglutinate. Unfortunately, when the agglutinated granulocytes were removed by centrifugation, a large number of the lymphocytes were also lost. With one of the leukocyte donors a lymphocyte suspension pure enough for T-cell subset and B-cell analysis was obtained. However, with the second donor this procedure failed to yield enough lymphocytes for analysis. The reasons behind the separation problem are unclear. The HES does not appear to be the cause, since the problem was encountered in the pre-apheresis specimens. Perhaps the large number of granulocytes present in the blood of these stimulated donors causes a "log jam" at the Ficoll-Hypaque interface and prevents the cells from

entering the medium. Other explanations include the possiblity that the pre-medication releases marginated granulocytes with a lower cell density, or changes the cell density of all of the granulocytes. In either case, these lower density graunlocytes would collect at the Ficoll-Hypaque interface along with the lymphocytes and prevent effective separation.

Superficially, it seems logical to expect higher lymphocyte losses in leukapheresis donors than in plateletpheresis donors since white blood cells are being harvested in the former. However, the use of pre-medications actually reduces the absolute number of lymphocytes present in the blood[42]. The steroid drugs cause a migration of the lymphocytes from the peripheral blood into the secondary lymphoid organs and thoracic duct.[43] Examination of the leukapheresis donors' absolute lymphocyte counts (Table 2c) demonstrates this effect. The absolute lymphocyte counts in these donors was below the normal levels (mean normal 2500 with a range of 1500 to 4000).[44] Consequently, the total lymphocyte loss in the two leukocyte donors studied was not excessive (Table 3c). In fact, some of the Haemonetics 30 plateletpheresis donors had higher lymphocytes losses (Table 3d). It would appear that the leukapheresis donors are not at any higher risk, and in fact may have a lower risk, with respect to lymphocyte removal since they have

fewer lymphocytes in their peripheral blood if premedication is used.

It is diffcult to evaluate the other results for the leukapheresis donors because of the small sample size. However, the T4, T8, T4:T8 ratio, percentage T3, and B-cell results do not appear to be significantly different from the values obtained for the plateletpheresis donors. However, any conclusions regarding the leukapheresis donor must be considered speculative because of the small sample of leukapheresis donors studied.

No statistically significant difference was observed between the donors over 40 years and under 40 years of age. If it is assumed that thymic involution is complete in the donors over 40, their mechanism for T-cell replacement is a concern. Apparently the pool of lymphocytes within the body is adequate for replacement of lymphocytes removed during apheresis. The ability of the lymphocyte pool to respond to repeated lymphocyte removal is unknown. Four of the donors in this study (#3, 9, 11, and 14) were over 40 years old and had undergone previous apheresis procedures. All experienced a post-apheresis decrease in absolute lymphocyte count. However, these decreases were well within the range observed for the other donors. One week follow-up data was available for two of the four donors. One donor (#9) showed a slight increase in absolute count in the one week sample while the other (#14) showed a slight drop. Further research would be

necessary to prove or disprove any adverse impact of apheresis on donors over 40 years of age.

The comparisons between donors with and without previous apheresis procedures showed no major difference in their response to the studied procedure. The question of how frequently donors may undergo apheresis remains unclear. With respect to lymphocyte removal, the type of apheresis instrument used may be the most critical factor since the instruments clearly differ in the number of lymphocytes removed per procedure. Guidelines for frequency of apheresis donation have been published by the Food and Drug Administration (FDA).[45] These guidelines require that a donor undergo no more than 12 apheresis procedures per 12 month period. Furthermore, the guidelines limit the number of procedures to two per week and six per month with at least a 48 hour interval between procedures. If we assume a loss of 5×10^9 lymphocytes per procedure on the Haemonetics 30, a donor could lose 1×10^{10} lymphocytes in 48 hours, 3×10^{10} within a month, and 6×10^{10} per year following the FDA guidelines. These losses are below the 1×10^{11} cell loss necessary to produce an immunosuppressive effect in arthritis patients.[6] The loss of 5×10^9 cells per procedure is higher than the loss observed in this study on the Haemonetics 30, and therefore provides a margin of safety in the estimates. As discussed previously, the lymphocyte loss

in the IBM 2997 and Haemonetics V-50 donors is significantly lower. The FDA guidelines would appear to be conservative for donors undergoing apheresis on these instruments, at least with respect to lymphocyte removal. However, lymphocyte loss should not be the only criteria used to establish permissable apheresis frequency. Other factors to be considered include platelet loss, red blood cell loss, plasma loss and serum immunoglobulin changes. In addition, exposure to steroid drugs and HES become considerations in leukapheresis donors. Furthermore, until the effect of B-cell removal can be fully investigated, the question of safe apheresis frequency can not be completely answered.

Conclusions

The pool of circulating lymphocytes is stressed when a donor undergoes apheresis. The B-cell portion of the pool appears to be reduced. The effect of this loss of B-cells on the donors' health is unknown. In this study, no significant changes in the ratio of helper to suppressor T-lymphocytes was observed. Apparently, the immune balance of regulatory lymphocytes is not upset by apheresis. Other effects of apheresis on the T-cell portion of the lymphocyte pool remain unclear.

Several factors can be identified which potentially influence the lymphocyte loss which occurs during apheresis. Among these are the donors' age, the number or frequency of apheresis donations, and the type of apheresis product being collected. In this study, none of these factors appeared to be significant in lymphocyte loss. However, significant differences were observed between different apheresis instruments. Furthermore, changes in the operational technique can also influence the lymphocyte loss. The IBM 2997 and Haemonetics V-50 instruments remove significantly fewer lymphocytes than the Haemonetics 30 instrument. The lymphocyte loss with the Haemonetics 30 instrument can be reduced by changing the collection technique. The use of the newer technology IBM 2997 or Haemonetics V-50 equipment can reduce the lymphocyte loss

during apheresis to a level within the range of the lymphocyte loss that occurs during whole blood donation. U_e of a modified collection technique on the older technology Haemonetics 30 instrument can produce similar results.

Nonetheless, it would appear prudent to limit the frequency of apheresis donations for any single donor until further research can be conducted on lymphocyte depletion. In particular, the significance of B-cell removal on the donors' long term health would merit further investigation. The published FDA guidelines for apheresis frequency would appear to be reasonable for Haemonetics 30 apheresis donors and conservative for IBM 2997 and Haemonetics V-50 donors with respect to lymphocyte depletion.

FIGURE 1

DONOR CONSENT FORM

Informed Consent Statement

Before agreeing to participate in this study, it is important that the following explanation of the proposed procedures be read and understood. It describes the purpose of the study. It also describes alternative procedures available and the right to withdraw from the study at any time. It is important to understand that no guarantee or assurance can be made as to the results. It is also understood that refusal to participate in this study will not influence standard treatment for the subject.

Objectives:

I,____________________________ agree to participate in a research study the purpose of which is to determine the effect of my apheresis donation on the numbers and types of white blood cells in my circulation.

Procedures:

Three test tubes of blood, approximately 25 milliliters, will be obtained from a vein in my arm before the apheresis procedure and immediately after completion of the procedure (total of 50 milliliters). In addition, the researcher will schedule an appointment for 7 to 10 days after the apheresis procedure to obtain another blood specimen (25 milliliters). The blood specimens will be used for laboratory tests to determine the numbers and types of white blood cells present. The results of these tests along with the results from other apheresis donors will be used to determine if any significant change occurs in white cell types after apheresis.

Risks:

The risks of simple venipunture include: commonly, the occurrence of discomfort and/or bruise at the site of puncture; and less commonly, the formation of a small blood clot or swelling of the vein and surrounding tissue, and bleeding from the puncture site.

FIGURE 1

PAGE 2

Confidentiality of records:

All records identifying the subject will be handled confidentially and all references to the subject will be by a coded number.

Availability of information:

Any questions that I may have cencerning any aspect of this investigation will be answered by:

Terry J. Meier, Graduate Student
Hoxworth Blood Center 569-1193
Home Phone 742-5259
-OR-
Kamala Balakrishnan, MD
Hoxworth Blood Center 569-1184

Compensation:

The University of Cincinnati Medical Center follows a policy of making all decisions concerning compensation and medical treatment for injuries occuring during or caused by participation in biomedical or behavioral research on an individual basis. If I believe I have been injured as a result of research, I will contact:

Terry J. Meier
Hoxworth Blood Center 569-1176 or 569-1193
Home Phone 742-5259

The right to withdraw:

I am free to withdraw from this investigation at any time. Should I wish to withdraw, I have been assured that standard therapy will remain available. I have been informed of the probable consequences of my withdrawal from the study.

Witnessing and signatures:

Subject:______________________________ Date:__________

Investigator:__________________________ Date:__________

Witness:______________________________ Date:__________

FIGURE 2

SUBJECTS AND APHERESIS PROCEDURES

Donor	Age/Sex	Procedure	Instrument Volume/Cycles	Previous Procedures
1(a)	52/M	Platelet	IBM 2997 2250 ml	None
2	29/M	Platelet	IBM 2997 2060 ml	6 Platelets
3	55/M	Platelet	IBM 2997 3330 ml	7 Platelets
4	21/M	Platelet	IBM 2997 3700 ml	1 Platelet
5	23/M	Platelet	IBM 2997 3630 ml	None
6	53/M	Platelet	IBM 2997 3320 ml	None
7	44/M	Platelet	Haemonetics V-50 6 cycles	None
8	32/M	Platelet	Haemonetics V-50 6 cycles	None
9	42/F	Platelet	Haemonetics V-50 6 cycles	1 Platelet
1(b)	52/M	Platelet	Haemonetics V-50 6 cycles	2 Platelets
10	52/M	Leuko. pre-Prednisone	Haemonetics 30 6 cycles	None
11	55/M	Leuko. pre-Dexamethasone	Haemonetics 30 6 cycles	2 Leuko.
12	22/F	Platelet RBC free	Haemonetics 30 8 cycles	None
13	25/M	Platelet	Haemonetics 30 6 cycles	1 Platelet
14	54/F	Platelet	Haemonetics 30 6 cycles	2 Platelets 1 Leuko.
15	33/F	Platelet	Haemonetics 30 6 cycles	None

FIGURE 3

LYMPHOCYTE LOSS WITH DIFFERENT APHERESIS INSTRUMENTS

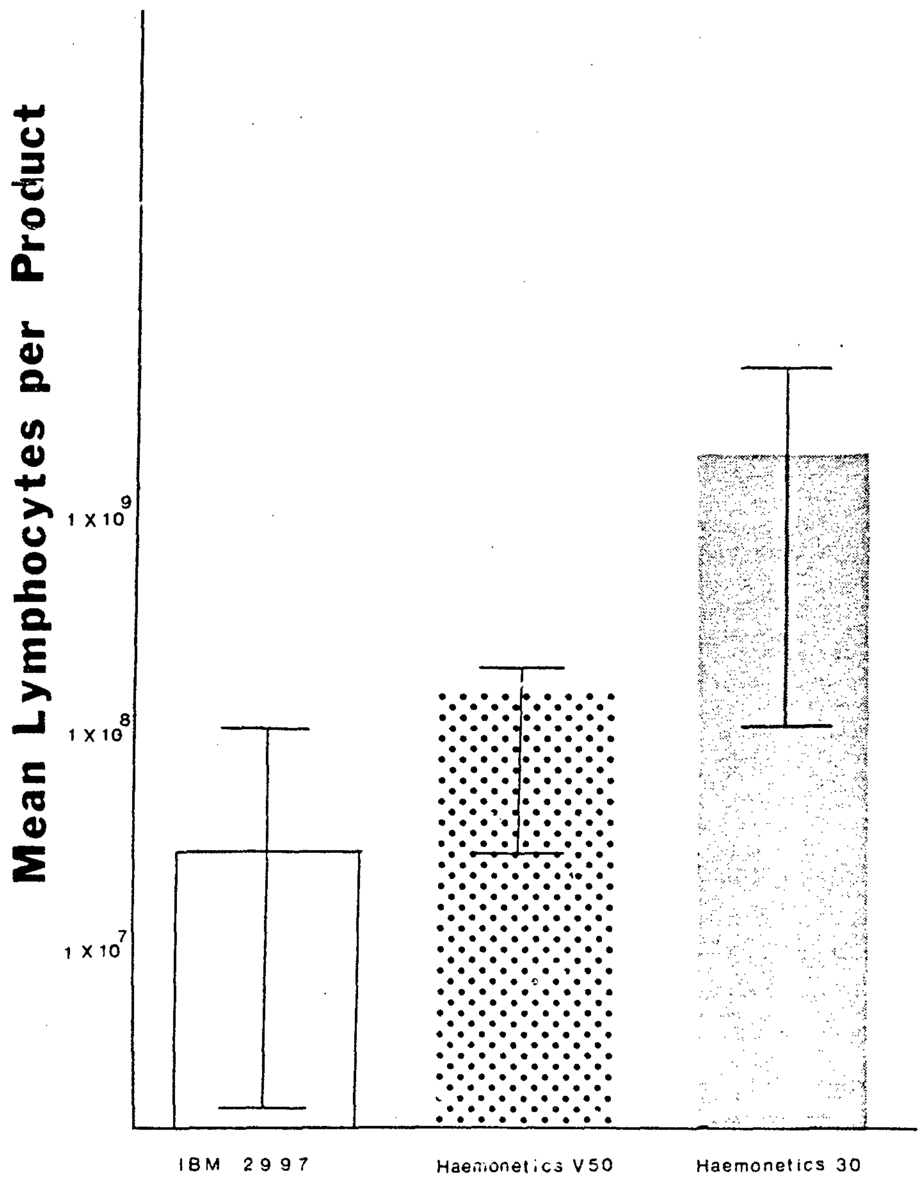

FIGURE 4

B-CELL RESULTS (ALL DONORS)

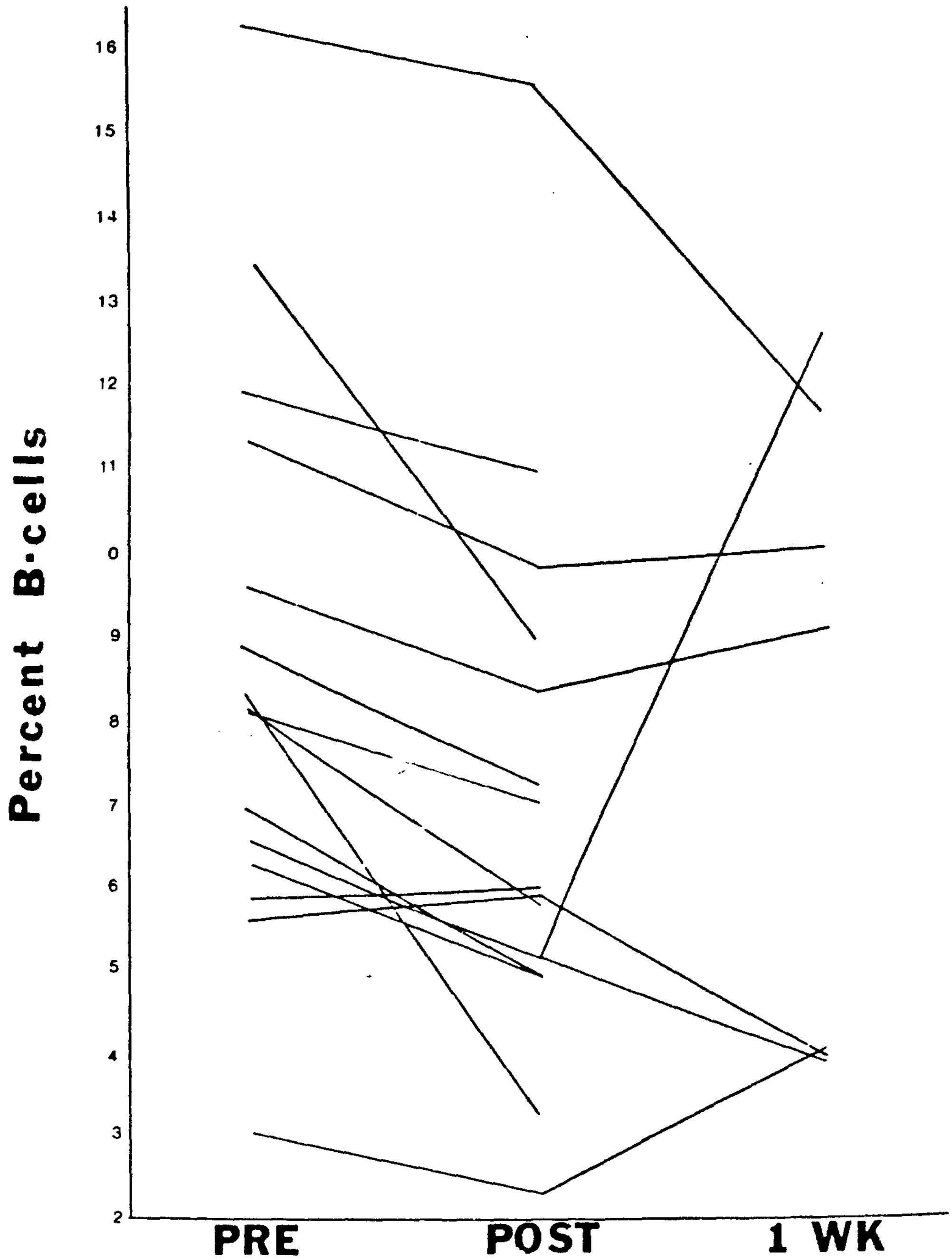

TABLE 1

T-CELL SUBSET AND B-CELL RESULTS ON NORMAL CONTROLS

	% T3	% T4	% T8	T4:T8	% B-CELLS
Mean	76	50	23	2.2	10
2 SD	11.5	8.0	3.8	1.1	7.9

TABLE 2a

IBM 2997 PLATELET DONORS RESULTS FOR TOTAL WHITE CELL COUNTS AND ABSOLUTE LYMPHOCYTE COUNTS (cells per mm^3)

	Total WBC Counts			Absolute lymphocytes		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
1(a)	3000	3900	NA	1400	1500	NA
2	7500	7000	NA	3500	4200	NA
3	7300	7300	NA	4200	3100	NA
4	5800	8200	6200	3000	2300	2900
5	11,100	7800	6000	4700	4300	2600
6	9400	9200	NA	2400	2200	NA
Mean	7400	7200	6100	3200	2900	2800
2 SD	5600	3600	200	2400	2200	400
Paired t test	Pre versus Post 0.150			Pre versus Post 1.040		
At p=0.05	n.s.			n.s.		

TABLE 2b

HAEMONETICS V-50 PLATELET DONORS RESULTS FOR

TOTAL WHITE CELL COUNTS AND ABSOLUTE LYMPHOCYTE COUNTS

(cells per mm^3)

	Total WBC Counts			Absolute lymphocytes		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
1(b)	6400	4300	3900	3300	2000	2000
7	12,500	9500	NA	8100	5700	NA
8	6800	7200	NA	2200	2000	NA
9	5600	5100	5300	4300	3400	3600
Mean	7800	6500	ND	4500	3300	ND
2 SD	6000	4600	ND	5000	3400	ND
Paired t test	Pre versus Post 1.695			Pre versus Post 2.608		
At p=0.05	n.s.			n.s.		

TABLE 2c

HAEMONETICS 30 LEUKOCYTE DONORS RESULTS FOR

TOTAL WHITE CELL COUNTS AND ABSOLUTE LYMPHOCYTE COUNTS

(cells per mm^3)

	Total WBC Counts			Absolute lymphocytes		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
10	6600	6200	5600	1500	400	2200
11	4400	4800	NA	1100	900	NA
Mean	5500	5500	ND	1300	650	ND
2 SD	3000	2000	ND	600	800	ND
Paired t test	Pre versus Post 0.000			Pre versus Post 1.444		
At p=0.05	n.s.			n.s.		

TABLE 2d

HAEMONETICS 30 PLATELET DONORS RESULTS FOR

TOTAL WHITE CELL COUNTS AND ABSOLUTE LYMPHOCYTE COUNTS

(cells per mm^3)

	Total WBC Counts			Absolute lymphocytes		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
12	4000	2900	6300	2500	1400	1800
13	11,400	9900	NA	2400	2200	NA
14	9300	8100	7600	4000	3400	2900
15	7600	6200	5300	2900	2600	2800
Mean	8100	6800	6400	3000	2400	2500
2 SD	6000	6000	2200	1400	1600	1200
Paired t test	Pre versus Post 14.240			Pre versus Post 2.722		
At p=0.05	Significant			n.s.		
Paired t test	Post versus 1 Week 0.486			Post versus 1 Week 0.122		
At p=0.05	n.s.			n.s.		

TABLE 2e

CUMULATIVE DONOR RESULTS FOR

TOTAL WHITE CELL COUNTS AND ABSOLUTE LYMPHOCYTE COUNTS

(cells per mm^3)

	Total WBC Counts			Absolute lymphocytes		
	Pre	Post	1 Week	Pre	Post	1 Week
Mean	7400	6700	5800	3200	2600	2600
2 SD	5400	4200	2100	3400	2700	1200
n	16	16	8	16	16	8

	Total WBC Counts	Absolute lymphocytes
Unpaired t test	Pre versus Post 0.809	Pre versus Post 1.139
At p=0.05	n.s.	n.s.
Unpaired t test	Post versus 1 Week 0.570	Post versus 1 Week 0.000
At p=0.05	n.s.	n.s.

TABLE 3a

IBM 2997 PLATELET PRODUCTS RESULTS FOR WHITE CELL COUNTS,

TOTAL WHITE CELLS, PERCENT LYMPHOCYTES,

AND TOTAL LYMPHOCYTES (counts in cells per mm^3)

Donor #	WBC Count	Total WBC's	Percent Lymphs	Total Lymphs
1(a)	1.0×10^2	1.4×10^7	68	9.5×10^6
2	NA	NA	NA	NA
3	NA	NA	NA	NA
4	6.1×10^2	1.6×10^8	49	7.8×10^7
5	1.2×10^2	3.5×10^7	75	2.6×10^7
6	4.0×10^2	8.4×10^7	85	7.1×10^7
Mean	3.1×10^2	7.3×10^7	69	4.6×10^7
2 SD	4.8×10^2	1.3×10^8	30	7.0×10^7

TABLE 3b

HAEMONETICS V-50 PLATELET PRODUCTS RESULTS

FOR WHITE CELL COUNTS, TOTAL WHITE CELLS,

PERCENT LYMPHOCYTES, AND TOTAL LYMPHOCYTES

(counts in cells per mm^3)

Donor #	WBC Count	Total WBC's	Percent Lymphs	Total Lymphs
1(b)	9.0×10^2	2.7×10^8	42	1.1×10^8
7	1.8×10^3	3.4×10^8	62	2.1×10^8
8	NA	NA	NA	NA
9	2.0×10^3	5.0×10^8	50	2.5×10^8
Mean	1.6×10^3	3.7×10^8	51	1.9×10^8
2 SD	1.2×10^3	2.0×10^8	20	1.4×10^8

TABLE 3c

HAEMONETICS V-50 LEUKOCYTE PRODUCTS RESULTS

FOR WHITE CELL COUNTS, TOTAL WHITE CELLS,

PERCENT LYMPHOCYTES, AND TOTAL LYMPHOCYTES

(counts in cells per mm^3)

Donor #	WBC Count	Total WBC's	Percent Lymphs	Total Lymphs
10	9.0×10^4	1.4×10^{10}	20	2.8×10^9
11	2.2×10^4	7.5×10^9	18	1.4×10^9
Mean	5.6×10^4	1.1×10^{10}	19	2.1×10^9
2 SD	9.6×10^4	0.9×10^{10}	ND	1.9×10^9

TABLE 3d

HAEMONETICS 30 PLATELET PRODUCTS RESULTS

FOR WHITE CELL COUNTS, TOTAL WHITE CELLS,

PERCENT LYMPHOCYTES, AND TOTAL LYMPHOCYTES

(counts in cells per mm^3)

Donor #	WBC Count	Total WBC's	Percent Lymphs	Total Lymphs
12	1.7×10^3	3.2×10^8	55	1.8×10^8
13	4.5×10^4	6.4×10^9	78	4.9×10^9
14	3.6×10^4	5.6×10^9	60	3.3×10^9
15	3.2×10^4	5.5×10^9	63	3.4×10^9
Mean	2.9×10^4	4.5×10^9	64	2.9×10^9
2 SD	3.8×10^4	5.6×10^9	20	4.0×10^9

TABLE 3e

COMPARISON OF TOTAL LYMPHOCYTES IN PLATELET PRODUCTS

FROM DIFFERENT APHERESIS INSTRUMENTS

Comparison	Unpaired t test	Significant at
IBM 2997 vs Haemonetics 30	2.923	p=0.05
IBM 2997 vs Haemonetics V-50	3.588	p=0.05
Haemonetics 30 vs Haemonetics V-50	2.373	p=0.10

TABLE 4a

IBM 2997 PLATELET DONORS T4 (HELPER), T8 (SUPPRESSOR),

AND T4:T8 RATIO RESULTS

Donor #	% T4 Pre/Post/1 Week			% T8 Pre/Post/1 Week			T4:T8 Pre/Post/1 Week		
1(a)	51	50	NA	28	30	NA	1.8	1.7	NA
2	60	56	NA	27	28	NA	2.2	2.0	NA
3	56	63	NA	18	24	NA	3.1	2.6	NA
4	46	50	49	28	28	33	1.6	1.8	1.5
5	54	48	53	23	21	21	2.4	2.3	2.5
6	51	53	NA	20	21	NA	2.6	2.8	NA
Mean	53	53	51	24	25	27	2.3	2.2	2.0
2 SD	10	11	6	9	8	17	1.1	0.8	1.4
Paired t test	Pre vs Post 0.166			Pre vs Post 1.229			Pre vs Post 0.773		
At p=0.05	n.s.			n.s.			n.s		

TABLE 4b

HAEMONETICS V-50 PLATELET DONORS T4 (HELPER), T8 (SUPPRESSOR), AND T4:T8 RATIO RESULTS

Donor #	% T4 Pre	% T4 Post	% T4 1 Week	% T8 Pre	% T8 Post	% T8 1 Week	T4:T8 Pre	T4:T8 Post	T4:T8 1 Week
1(b)	43	53	47	24	19	24	1.8	2.8	1.9
7	53	55	NA	17	23	NA	3.1	2.4	NA
8	50	45	NA	21	20	NA	2.4	2.3	NA
9	47	42	44	29	23	21	1.6	1.8	2.1
Mean	48	49	46	23	21	23	2.2	2.3	2.0
2 SD	8	12	4	10	4	4	1.3	0.8	0.3
Paired t test	Pre vs Post 0.140			Pre vs Post 0.551			Pre vs Post 0.283		
At p=0.05	n.s.			n.s.			n.s		

TABLE 4c

HAEMONETICS 30 LEUKOCYTE DONORS T4 (HELPER), T8 (SUPPRESSOR), AND T4:T8 RATIO RESULTS

Donor #	% T4 Pre/Post/1 Week			% T8 Pre/Post/1 Week			T4:T8 Pre/Post/1 Week		
10	NA	NA	49	NA	NA	21	NA	NA	2.3
11	55	51	NA	20	23	NA	2.8	2.2	NA

TABLE 4d

HAEMONETICS 30 PLATELET DONORS T4 (HELPER),

T8 (SUPPRESSOR), AND T4:T8 RATIO RESULTS

Donor #	% T4 Pre/Post/1 Week			% T8 Pre/Post/1 Week			T4:T8 Pre/Post/1 Week		
12	51	52	48	21	20	21	2.4	2.6	2.3
13	49	46	NA	21	21	NA	2.3	2.2	NA
14	52	51	46	22	21	22	2.4	2.4	2.0
15	48	51	48	22	23	20	2.2	2.2	2.4
Mean	50	50	47	22	21	21	2.3	2.4	2.2
2 SD	4	6	2	1	2	2	0.2	0.4	0.4
Paired t test	Pre vs Post 0.000			Pre vs Post 0.522			Pre vs Post 0.397		
At p=0.05	n.s.			n.s.			n.s		
Paired t test	Post vs 1 Week 2.310			Post vs 1 Week 0.250			Post vs 1 Week 0.898		
At p=0.05	n.s.			n.s.			n.s.		

TABLE 4e

CUMULATIVE T4 (HELPER), T8 (SUPPRESSOR), AND T4:T8 RATIO

RESULTS FOR ALL APHERESIS DONORS

	% T4 Pre	% T4 Post	% T4 1 Week	% T8 Pre	% T8 Post	% T8 1 Week	T4:T8 Pre	T4:T8 Post	T4:T8 1 Week
Mean	51	51	48	23	23	23	2.3	2.3	2.1
2 SD	9	10	6	8	7	9	0.9	0.7	0.6
n	15	15	8	15	15	8	15	15	8

	% T4	% T8	T4:T8
Unpaired t test	Pre vs Post 0.000	Pre vs Post 0.208	Pre vs Post 0.264
At p=0.05	n.s.	n.s.	n.s
Unpaired t test	Post vs 1 Week 1.799	Post vs 1 Week 0.786	Post vs 1 Week 1.000
At p=0.05	n.s.	n.s.	n.s.

TABLE 5a

IBM 2997 PLATELET DONORS PERCENT T-CELLS (BY % T3 POSITIVE)

AND ABSOLUTE T-CELL COUNTS (cells per mm^3)

	% T3			Absolute T-cells		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
1(a)	78	77	NA	1100	1200	NA
2	88	87	NA	3100	3700	NA
3	80	81	NA	3400	2500	NA
4	85	83	74	2600	1900	2100
5	78	76	72	3700	3300	1900
6	73	70	NA	1800	1500	NA
Mean	80	79	73	2600	2300	ND
2 SD	11	12	3	2000	2000	ND
Paired t test	Pre versus Post 2.390			Pre versus Post 1.195		
At p=0.05	n.s.			n.s.		

TABLE 5b

HAEMONETICS V-50 PLATELET DONORS

PERCENT T-CELLS (BY % T3 POSITIVE)

AND ABSOLUTE T-CELL COUNTS (cells per mm^3)

Donor #	% T3 Pre	% T3 Post	% T3 1 Week	Absolute T-cells Pre	Absolute T-cells Post	Absolute T-cells 1 Week
1(b)	78	76	85	2600	1500	1700
7	79	83	NA	6400	4700	NA
8	76	79	NA	1700	1600	NA
9	69	70	68	3000	2400	2500
Mean	76	77	77	3400	2500	ND
2 SD	10	11	24	4000	3000	ND
Paired t test	Pre versus Post 1.134			Pre versus Post 2.555		
At p=0.05	n.s.			n.s.		

TABLE 5c

HAEMONETICS 30 LEUKOCYTE DONORS

PERCENT T-CELLS (BY % T3 POSITIVE)

AND ABSOLUTE T-CELL COUNTS (cells per mm^3)

	% T3			Absolute T-cells		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
10	NA	NA	63	NA	NA	1400
11	72	71	NA	790	640	NA

TABLE 5d

HAEMONETICS 30 PLATELET DONORS

PERCENT T-CELLS (BY % T3 POSITIVE)

AND ABSOLUTE T-CELL COUNTS (cells per mm^3)

Donor #	% T3 Pre	% T3 Post	% T3 1 Week	Absolute T-cells Pre	Absolute T-cells Post	Absolute T-cells 1 Week
12	80	80	78	2000	1100	1400
13	68	78	NA	1600	1700	NA
14	77	79	69	3100	2700	2000
15	70	66	71	2000	1700	2000
Mean	74	76	72	2200	1800	ND
2 SD	12	12	10	1300	1300	ND
Paired t test	Pre versus Post 0.679			Pre versus Post 1.823		
At p=0.05	n.s.			n.s.		
Paired t test	Post versus 1 Week 0.570			Post versus 1 Week 0.100		
At p=0.05	n.s.			n.s.		

TABLE 5e

CUMULATIVE PERCENT T-CELLS (BY % T3 POSITIVE)

AND ABSOLUTE T-CELL COUNTS (cells per mm^3)

FOR ALL APHERESIS DONORS

	% T3			Absolute T-cells		
	Pre	Post	1 Week	Pre	Post	1 Week
Mean	77	77	72	2600	2100	1900
2 SD	11	12	13	2800	2200	700

	% T3	Absolute T-cells
Unpaired t test	Pre versus Post 0.160	Pre versus Post 1.003
At p=0.05	n.s.	n.s.
Unpaired t test	Post versus 1 Week 1.753	Post versus 1 Week 0.668
At p=0.05	n.s.	n.s.

TABLE 6a

IBM 2997 PLATELET DONOR B-CELL RESULTS

	% B-cells			Absolute B-cells		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
1(a)	5.8	5.9	NA	81	88	NA
2	8.9	7.1	NA	311	298	NA
3	8.1	7.0	NA	340	217	NA
4	5.7	5.6	3.9	170	129	113
5	16.3	15.6	11.5	766	406	299
6	7.8	5.8	NA	187	128	NA
Mean	8.8	7.8	ND	309	211	ND
2 SD	7.8	7.7	ND	480	240	ND
Paired t test	Pre versus Post 2.645			Pre versus Post 1.769		
At p=0.05	Significant			n.s.		

TABLE 6b

HAEMONETICS V-50 PLATELET DONOR B-CELL RESULTS

Donor #	% B-cells Pre	Post	1 Week	Absolute B-cells Pre	Post	1 Week
1(b)	3.0	2.3	4.0	99	46	80
7	6.9	4.4	NA	559	251	NA
8	11.9	11.1	NA	262	222	NA
9	11.3	9.9	10.0	512	337	360
Mean	8.3	6.9	ND	360	210	ND
2 SD	8.4	8.4	ND	430	240	ND
Paired t test	Pre versus Post 3.266			Pre versus Post 2.302		
At p=0.05	Significant			n.s.		

TABLE 6c

HAEMONETICS 30 LEUKOCYTE DONOR B-CELL RESULTS

	% B-cells			Absolute B-cells		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
10	NA	NA	8.0	NA	NA	176
11	8.0	2.9	NA	88	26	NA

TABLE 6d

HAEMONETICS 30 PLATELET DONOR B-CELL RESULTS

	% B-cells			Absolute B-cells		
Donor #	Pre	Post	1 Week	Pre	Post	1 Week
12	6.2	5.2	4.0	155	73	72
13	13.4	8.8	NA	322	194	NA
14	6.5	5.0	12.5	260	170	363
15	9.6	8.4	8.90	278	218	250
Mean	8.9	6.9	8.5	250	160	230
2 SD	6.7	4.1	8.5	140	120	300
Paired t test	Pre versus Post 2.447			Pre versus Post 6.353		
At p=0.05	n.s.			Significant		
Paired t test	Post versus 1 Week 2.662			Post versus 1 Week 1.246		
At p=0.05	n.s.			n.s.		

TABLE 6e

CUMULATIVE B-CELL RESULTS FOR ALL APHERESIS DONORS

	% B-cells			Absolute B-cells		
	Pre	Post	1 Week	Pre	Post	1 Week
Mean	8.6	7.0	7.9	293	187	214
2 SD	6.8	6.8	15.0	400	210	240
Unpaired t test	Pre versus Post 1.307			Pre versus Post 1.854		
At p=0.05	n.s.			n.s.		
Paired t test	Post versus 1 Week 0.565			Post versus 1 Week 0.552		
At p=0.05	n.s.			n.s.		

TABLE 7a

RESULTS FOR APHERESIS DONORS OVER 40 YEARS

AND UNDER 40 YEARS

Donors under 40 years (n=7)

	% T3 Pre/Post		% T4 Pre/Post		% T8 Pre/Post		T4:T8 Pre/Post		B-cells Pre/Post	
Mean	78	78	51	50	23	23	2.2	2.2	10.3	8.8
2 SD	14	13	9	8	6	8	0.6	0.5	7.6	7.2
Unpaired t test (Pre vesrus Post)	0.154		0.632		0.163		0.099		0.732	
At p=0.05	n.s.		n.s.		n.s.		n.s.		n.s.	

Donors over 40 years (n=8)

	% T3 Pre/Post		% T4 Pre/Post		% T8 Pre/Post		T4:T8 Pre/Post		B-cells Pre/Post	
Mean	76	76	51	52	22	23	2.4	2.3	7.2	6.4
2 SD	8	10	8	11	8	7	1.2	0.8	4.7	4.9
Unpaired t test (Pre vesrus Post)	0.055		1.472		0.386		0.241		0.679	
At p=0.05	n.s.		n.s.		n.s.		n.s.		n.s.	

TABLE 7b

COMPARISON OF POST-APHERESIS RESULTS BETWEEN

UNDER 40 YEAR (u40) AND OVER 40 (o40) YEAR GROUPS

	% T3		% T4		% T8		T4:T8		B-cells	
	u40	o40	u40	o40	u40	o40	u40	o40	u40	o40
Mean	78	76	51	51	23	23	2.2	2.3	6.4	8.8
Unpaired t test	0.849		0.983		0.000		0.758		1.566	
At p=0.05	n.s.		n.s.		n.s.		n.s.		n.s.	

TABLE 8a

RESULTS FOR APHERESIS DONORS WITH AND WITHOUT

PREVIOUS APHERESIS DONATIONS

Donors with no previous donations (n=7)

	% T3 Pre/Post		% T4 Pre/Post		% T8 Pre/Post		T4:T8 Pre/Post		B-cells Pre/Post	
Mean	76	76	51	51	22	22	2.4	2.3	9.2	8.1
2 SD	7	12	4	7	7	7	0.4	0.4	3.7	4.0
Unpaired t test (Pre vesrus Post)	0.164		0.394		0.468		0.433		0.554	
At p=0.05	n.s.		n.s.		n.s.		n.s.		n.s.	

Donors with previous donations (n=8)

	% T3 Pre/Post		% T4 Pre/Post		% T8 Pre/Post		T4:T8 Pre/Post		B-cells Pre/Post	
Mean	77	78	51	52	24	23	2.2	2.2	8.1	6.1
2 SD	14	11	11	12	8	6	1.1	0.7	6.5	5.3
Unpaired t test (Pre vesrus Post)	0.306		0.166		0.137		0.000		1.375	
At p=0.05	n.s.		n.s.		n.s.		n.s.		n.s.	

TABLE 8b

COMPARISON OF POST-APHERESIS RESULTS BETWEEN DONORS WITH AND WITHOUT (W/O) PREVIOUS APHERESIS DONATIONS

	% T3		% T4		% T8		T4:T8		B-cells	
	With	W/O	With	W/O	With	W/O	With	W/O	With	W/O
Mean	77	76	52	51	23	23	2.2	2.3	6.1	8.1
Unpaired t test	0.750		0.349		0.461		0.565		1.138	
At p=0.05	n.s.		n.s.		n.s.		n.s.		n.s.	

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